Article 13 Report Maize and Biodiversity: The Effects of Transgenic Maize in Mexico
Chapter 4. Assessment of effects on natural ecosystems
L. LaReesa Wolfenbarger and Mario González-Espinosa

Maize and Biodiversity: The Effects of Transgenic Maize in Mexico

Chapter 4
Assessment of Effects on Natural Ecosystems

for the Article 13 Initative on
Maize and Biodiversity

Prepared by Lillian LaReesa Wolfenbarger and Mario González-Espinosa

Advisory Group Reviewers:
Peter Raven and José Sarukhán

External Reviewers:
Angelika Hilbeck, Deborah Letourneau, Daniel Piñero and Garrison Wilkes

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Abstract

Assessing effects of adopting transgenic maize on natural ecosystems in Mexico depends on obtaining information that can reliably detect changes in natural ecosystems in Mexico and on comparing such information to existing baseline agricultural practices in Mexico. Appropriate baseline comparisons in Mexico will vary due to the diversity of production systems that exist. Similarly, the choice of what indicators to use to detect changes in natural ecosystems will vary due to the diversity of ecosystems in Mexico. Although different ecosystems have general similarities in function (e.g., nutrient cycling, energy flow) and categories of goods and services (water purification, decomposition, pest control, pollination, foods, fuel, fibers and drugs), the specific factors or combinations of factors that affect ecosystem function may vary regionally depending on characteristics of biodiversity. At least four factors associated with biodiversity may affect ecosystems: what species are present, how many individuals of each species, how a species functions within the ecosystem (e.g., energy producer (photosynthetic plant), energy consumer (herbivore, predator, decomposer), and what biological interactions affect species' function(s) within the ecosystem. An assessment of effects of transgenic maize on natural ecosystems must recognize the general uncertainty in predicting the consequences of alterations to populations, species or communities upon ecosystems.

Mexico's biological richness is widely recognized as one of the largest in the world. Paralleling this, Mexico has a rich cultural diversity and ancient agricultural history. It is currently recognized as center of origin for as many as 100 crops from which fruit, seeds, roots, condiments, tubers, drugs, textiles, dyestuffs, resins, ornamental plants, and other natural products or services are derived. Taken together, the considerable diversity of biological species, communities, and ecosystems; of maize landraces; of culture and of agricultural practices underscores the importance of defining the scale of an assessment. The impacts of introducing transgenic maize have the potential to vary considerably according to any combination of factors associated with biological organization, maize genetic composition, culture or agricultural practices.

Potential effects on natural ecosystems of introducing transgenic maize should be assessed using comparisons with the baseline conditions of local or regional agriculture that would be replaced. Effects on natural ecosystems may arise if adoption of transgenic maize alters the abundance of individuals and if those alterations have resulting effects on population, species or communities. Effects on individuals of a species could arise if the transgenic organism is toxic and produces lethal or sublethal effects on individuals. Technological changes associated with the use of the transgenic organism (i.e., abandonment of some traditional farming practices) could also have effects on individual organisms that could cascade into higher order interactions. Alternatively, transgenic maize and its associated farming practices may have fewer effects on natural ecosystems than the existing agricultural practices of a given region. Higher levels of ecological organization and interactions are critical to ecosystems; therefore, relying on experiments focused at quantifying individual effects will underestimate any effects on natural ecosystems of introducing transgenic maize. Furthermore, small sample sizes and few replicates undermine the power of experiments to detect differences and will also produce underestimates of any effects on natural ecosystems.

The commercialization in the United States of Bt corn with insecticidal properties to control pests related to butterflies and moths has produced concerns about impacts on non-target butterflies and moths, as well as other insects that may ingest corn or corn pollen containing Bt toxin. Effects of Bt corn pollen dispersal on the three species of non-target butterflies studied so far suggest no adverse effects associated with currently available transgenic events (MON 810 and Bt11). Bt toxin in these transgenic events is toxic at high doses, but in the field the amount of Bt pollen encountered by the three species is considered below a level that could cause negative impacts. Prey-mediated effects (effects on insect predators that eat prey items that have fed on Bt plants or plants with Bt corn pollen) have been studied in two main taxa: the green lacewing (*Chrysoperla carnea*) and minute pirate bugs (*Orius* spp). Effects on individuals vary according to prey items. Where population level effects have been studied, no differences between Bt corn and non-Bt cornfields have been detected.

The applicability of this literature to assess natural ecosystem effects of introducing transgenic maize in Mexico has several limitations. First, recently compiled lists of potential species underscore the fact that a relatively small percentage of butterflies in the United States have been studied. How representative they are of Mexican biodiversity and natural ecosystems is tentative. Butterfly diversity in Mexico is substantially higher than in the United States, and from the existing data, it is clear that species sensitivity varies. Therefore, research on Mexican species will address the potential for impacts on butterfly and moth populations in Mexico. Similarly, predicting or testing effects on populations within natural ecosystems in Mexico will need experiments designed to consider valued or important Mexican species. Given the high degree of local and regional biodiversity, these valued and important species are likely to vary according to natural ecosystem, by culture, and by agricultural practices.

Equally important to assessing impacts on biodiversity and on natural ecosystem is quantifying and predicting how the introduction of transgenic maize may alter farming practices and landscapes and what impact, if any, these would have on natural ecosystems that interact with or intergrade into agro-ecosystems. Again, what changes may occur will depend on the transgenic event, the transgenic trait, adoption rates, and whether the transgenic maize also affects species important for agriculture (e.g., pollinators, pests). From recent work conducted in the United Kingdom, changes in farming practices associated with using herbicide-tolerant oilseed rape, beets or corn had impacts on insect and plant populations and diversity.

Our chapter highlights the need for further research and consideration of the link between biodiversity and ecosystem functioning and also the need for determining what magnitude of effects on individuals or populations is desirable to detect in order to predict higher order ecological effects. Also important will be identifying and understanding aspects of biodiversity and ecosystem functioning that are highly valued by those parties affected by the introduction of transgenic maize. We recognize that studying and quantifying all possible effects on natural ecosystems are not possible. As a high priority, we urge the use of data that reflect species and ecological functions of value at a regional scale. Therefore, case studies from North America or elsewhere are not easily generalized or applicable to Mexican biodiversity or ecosystems. In order to assess consequences of transgenic maize on biodiversity and on natural ecosystems, species within the areas of Mexico where the introduction of transgenic maize may occur must be studied.

Introduction

The goal of our chapter is to provide an assessment of how transgenic corn, and transgenic organisms in general, may affect natural ecosystems in Mexico, with particular focus on the region from where interest in this report initiated (Oaxaca). We define natural ecosystems as those non-agricultural ecosystems that occur largely without human intervention at the margins of agricultural fields or within larger tracts of land with less human disturbance. Therefore, in our definition, natural ecosystems are not necessarily pristine, undisturbed ecosystems, but also would include ecosystems interconnected with those managed intensively by humans and agriculture. The scientific basis for hypothesizing that genetically-engineered plants could have an effect on biodiversity stems from work from other fields of ecology that study biological introductions (e.g., invasion biology, island biogeography, biological control, species reintroductions). Any effects on biodiversity will be driven by the trait(s) engineered and their interactions with natural ecosystems. To the extent that genetic engineering enhances the diversity of traits available for current and future transgenic maize products, one can argue that genetic engineering itself has much to do with the potential for environmental effects (positive or negative).

Ecosystems and their biological functions

Ecosystems represent a collection of biological organisms, their dynamics as well as the abiotic features that affect organisms and processes (**Figure 1**). Ecosystems provide tangible and intangible, short-term and long-term functions as well as services of value to humans and other organisms, including pollination, air and water purification, decomposition, pest control, seed dispersal, nutrient cycling and generation, fertilization and preservation of soils (Daily et al. 1997). Studies on ecosystem consequences of biodiversity loss agree that a relationship between biodiversity and ecosystem function exists (Chapin et al. 1997, Loreau et al. 2001). Changes in biodiversity may affect ecosystems through at least four factors: (1) the abundance of each species, (2) the composition of species, (3) how a species functions in an ecosystem, and (4) biotic interactions that affect the magnitude and variability of a species' function(s) (Symstad et al. 2003); however, predicting the outcome of changes in biodiversity on these four factors remains unclear and under study (Naeem and Wright 2003). More specifically, determining what species have a significant impact on which function and what minimum number of species is required for ecosystem functioning and stability in changing environments remain unanswered.

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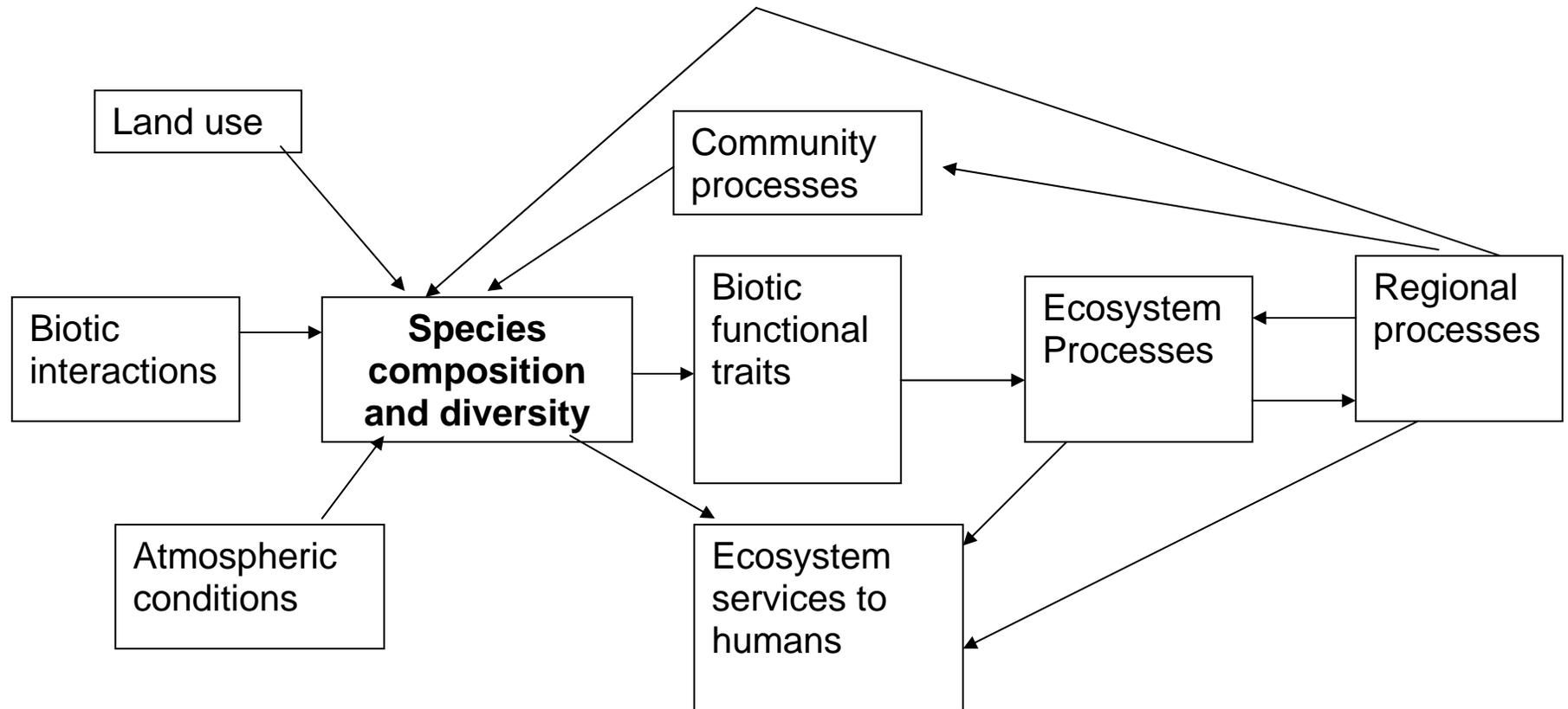**Figure 1.** Linkages among species diversity and ecosystem processes and ecosystem services to humans (after Chapin et al. 1997)

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Losses of biodiversity are widespread and are projected to continue (Naeem et al. 1999). Changes in land use and introduction of biological organisms (e.g. exotic species) are predicted to be potent drivers of global changes in biodiversity within terrestrial ecosystems in the 21st century (Sala et al. 2000). The introduction of genetically engineered organisms intersects these two areas of change. As biological organisms, they share with other introduced organisms the characteristic that they reproduce, disperse, and evolve in response to selection from biotic and abiotic factors. As a product used in agriculture, they may drive changes in land use that in turn affect biodiversity.

Biodiversity in Mexico

The biological richness of Mexico is widely recognized as one of the largest in the world (Ramamoorthy et al. 1993, Mittermeier 1988). Its biological richness results from great habitat variation and diverse ecological regions resulting from complex topography, heterogenous climate, geology, and geographical location. Mexico connects the Nearctic and the Neotropical biogeographical regions and is a site through which exchanges between the northern climates and tropical regions occurs (Halffter 1976, Rzedowski 1978). In particular the states of Oaxaca, Chiapas, Michoacán and Guerrero have high biological and cultural diversity (Ramamoorthy et al. 1993, Lorence and García-Mendoza 1989). As suggested for other areas of the globe (Mace and Pagel 1995, Moore et al. 2002, and other references therein), correlated patterns of cultural and biological diversity have also been observed in Mexico. Bye (1993) reports that more than 8 million people speak one of at least 54 native languages in a country harboring ca. 30,000 vascular plant species. According to different criteria for the identification and classification of plant formations, this enormous floristic diversity can be assembled in a large number of vegetation types. Flores Mata et al. (1972) identified at least 25 primary vegetation types for the whole country; Rzedowski (1978) developed a classification, mostly based on phytogeographical criteria, separating at least 11 major groups including up to 28 types; Miranda and Hernández Xolocotzi (1963) proposed a system including at least 32 basic types based on physiognomical criteria, and González Quintero (1974) proposed a classification of Mexican vegetation types with 9 major groups and up to 65 lower-level types. Therefore, it is not surprising that the coevolutionary interaction of humans with such a huge source of plant resources occurring over several millennia (Rindos 1984) resulted in the domestication of many modern cultivated plants. It is currently recognized that Mexico may be the center of origin of ca. 100 crops, including herbaceous, shrubby or tree species from which fruits, seeds, roots, tubers, condiments, drugs, textiles, dyestuffs, resins, ornamental plants, and other natural products or services are obtained (Hernández Xolocotzi 1985a, 1993, Challenger 1998).

Maize in Mexico

Mexico is the center of origin of maize (*Zea mays* ssp. *mays*), and its genetic diversity has been claimed to be one of the country´s greatest heritages (Brush et al. 1988). Recent molecular evidence suggests that maize most probably originated about 9,000 years ago through a single domestication event from the annual wild-grass teosinte *Zea mays* ssp. *parviglumis* in the candidate region of the central Balsas River basin of southern Mexico, below 1,800 m elevation

(Matsuoka et al. 2002). In addition, the findings of this recent study support a model based on archaeological records from 6,250 years ago, suggesting early diversification of maize in the highlands before spreading over the Americas along two dispersal paths (Piperno and Flannery 2001). One traces through western and northern Mexico into the southwestern United States. A second path leads out of the Mexican highlands to the western and southern lowlands of Mexico into Guatemala, the Caribbean Islands, the South American lowlands, and finally, the Andes. Recent analyses of DNA from archaeological materials indicate that early in the history of maize farmers selected for both morphological and biochemical traits (Jaenicke-Després et al. 2003) related to plant architecture and preparation of dough for *tortillas*.

Maize is the most widely grown cereal in the world, reflecting its ability to adapt to a wide range of agricultural habitats (Morris 2001). Already in pre-Columbian times its distribution ranged from eastern Canada to northern Chile (Matsuoka et al. 2002). For a number of reasons, maize is the most important food crop grown in Mexico. It is currently grown under a variety of ecological conditions, including rain fed and irrigated agricultural lands in many different climates: temperate humid, temperate semiarid, semi-warm humid, warm humid, warm sub-humid, and warm arid (Hernández Xolocotzi 1985a). A key climatic factor for this crop is that frost, or the short mid-summer drought, is not severe enough to interrupt its phenological development. Both locally derived landraces and commercial cultivars resulting from plant breeding programs are grown in tropical latitudes of Mexico, from sea level up to highland valleys at 2,800 m elevation located along the Sierra Madre Occidental, the Sierra Madre Oriental, the Transverse Volcanic Belt, the Sierra Madre del Sur in Oaxaca and Chiapas, and the Highlands of Chiapas (Hernández Xolocotzi 1985b, Perales et al. 2003).

Effects of agriculture on natural ecosystems and biodiversity

The introduction of agriculture into a natural landscape implies severe human alterations of the original ecosystems (Matson et al. 1997). Habitat fragmentation changes the relative extension of original habitats, may create novel environmental conditions at both local and landscape spatial scales (Saunders et al. 1991, Pickett and Cadenasso 1995), and may affect processes occurring in, and among, its component patches (Miles 1987, Turner 1989). Perhaps the largest scale effects that agriculture has on biodiversity occur within the field as an entire community of organisms is replaced with agricultural crops and associated organisms. From 1980 to 2001 the percentage of land conversion from each of seven vegetation cover types to man-made land cover (i.e., crops, exotic forest plantations, improved grasslands for livestock production and human settlements) ranged from 4 to 30% in Oaxaca (Vélazquez et al. 2003). Similar trends have been observed over the last four decades in the densely populated Mayan region of the central highlands of Chiapas (Wagner 1962, Ochoa-Gaona and González-Espinosa 2000, Ochoa-Gaona 2001). As the proportion of deforested land increases, natural ecosystems may be affected by agriculture at the field margins where boundaries abut and at distances where ecological interactions are possible through pollen, seed or animal dispersal, or through movement of water, nutrients or soil. Natural ecosystems may be subject to environmental stressors associated with agriculture, such as agro-chemical drift or leaching; disturbance and colonization by exotic species; or changes in habitat continuity (National Academy of Sciences 2001).

Agricultural landscapes in Mexico

The diversity of the patchwork of agricultural and non-agricultural habitats may be very different from one region to another due to variation in practices associated with maize production in Mexico. An assessment of effects of transgenic maize on natural ecosystems should incorporate how agricultural landscapes may change in their long-term configuration as a result of widespread and intensive use of transgenic maize, including major social and economic processes and forces, and predict what effect these changes may have on natural ecosystems.

Maize is currently produced in Mexico in a variety of farming systems (Hernández Xolocotzi 1985a). For current purposes, and for the sake of simplicity, they can be conveniently grouped under two extreme sets of production systems: extensive traditional and intensive modern (Table 1). Each group features its own production purposes, social and economical bases and consequences, genetic materials and diversity, agricultural practices, diversity of insect pests and diseases, diversity of associated plants (weeds and other tolerated and useful plants), inputs of materials and labor, social organization, and landscape structure. However, it should be noted that some systems that could be regarded as traditional are also quite intensive. In fact, at the time of the Spanish Conquest, at least eight agroecosystems based on maize could be identified, including the relatively extensive traditional system of slash-and-burn *milpa* agriculture, as well as a number of considerably more intensive farming systems (Coe 1964, Wilken 1969). These intensive systems, like the highland and lowland *chinampas*, used an extremely high diversity of crop plants and other organisms; drainage and irrigation practices to manipulate the growing season; manipulation of soil fertility through management of agricultural and other residues and soil conservation practices (Coe 1964, Harrison and Turner II 1978, Turner II and Harrison 1983). Many farming systems inspired by these ancient ones are still practiced to some extent (Wilken 1969, Hernández Xolocotzi 1985a, Zizumbo et al. 1988). However, a shortage of labor due to semi-proletarianization and emigration from Mexican rural communities have degraded the required underlying indigenous institutions, environmental conditions, and technological capacities (García-Barrios and García Barrios 1990, Pool-Novelo 1999).

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Table 1. Proposed contrasts in some major attributes between two extreme groups of the most extended agricultural systems in which maize is currently produced in Mexico. Many local systems that include a mixture of attributes can be recognized, but are obviated for simplicity.

Attribute	Extensive Traditional Farming Systems	Intensive Modern Farming Systems
Main purpose/objective	Self-consumption as a staple food for self-sufficiency. Other plants are maintained in the field for a variety of uses.	Market-driven cash-crops, usually for export from origin locality.
Origin of seed	Locally produced; result of non-formal local long-term selection on open pollinated landraces.	Modern open pollinated varieties and hybrids are used, which are the result of formal plant breeding programs (Morris 2001).
Local plant diversity	Relatively high; several open pollinated landraces of maize and a number of other crops and tolerated weeds or "agrestic" (*arvenses*) are typically used according to sowing calendars, purposes, climatic and soil conditions, with supposed beneficial effects through reducing output risks (Soto-Pinto 1997, Altieri 1999, Vieyra-Odilón and Vibrans 2001).	Relatively low; typically monoculture of maize hybrids with only a few commercially available cultivars used for mostly one purpose.
Agricultural practices	Numerous and relatively complex, aimed to benefit both the main crop species and the highly diverse weed flora (Bye 1981, 1993, Vieyra-Odilón and Vibrans 2001).	Relatively few and simple, only aimed to eradicate weed flora or decrease their detrimental effects through competition with main crop species.
Pathogens	Possibly high diversity; but widespread infections are possibly rare and with low effect on crop yields (Altieri 1999).	Low diversity; potential high risk of large effects on crop yields.

Table 1. Continued.

Attribute	Extensive Traditional Farming Systems	Intensive Modern Farming Systems
Use of modern machinery	Little; mostly dependent on human labor and animal tracking; widespread use of household-made or local-made tools (Warman 1988).	Mostly based on extensive mechanization, powered by electricity and/or fossil fuels.
Social organization	Contracts of diverse type, both formal and informal, but usually involving local workers; cooperation at family and community level may occur (Warman 1988, García-Barrios and García-Barrios 1990).	Mostly formalized contracts with paid workers; they may proceed from distant localities, and may not be familiar with local species and farming systems. (García-Barrios and García-Barrios 1990).
Plot size	Generally small; 77% of the land planted with maize in Mexico is < 5 ha (Turrent-Fernández et al. in OECD 2003); yet, in some areas a typical plot size is < 1 ha (e.g., Parra Vázquez 1989).	Generally large (> 5 ha).
Agricultural landscape structure	Relatively complex due to heterogeneous topography and interspersion of a variety of agricultural plots with secondary communities (different-aged fallow fields, pastures, secondary forests; e.g. Quintana-Ascencio et al. 1996, Ochoa-Gaona and González-Espinosa 2000). In addition, human landscape modifications allowing sustainable maize production in otherwise marginal habitats (e.g. irrigation of rainfed areas, drainage, slope terracing; García-Barrios and García-Barrios 1990, Pool-Novelo 1997, 1999).	Relatively simple and flat. Even if different types of patches are present, the landscape may be characterized by a relatively ordered pattern of patches, linear features such as roads, hedgerows, fences, irrigation channels, and other elements (Olson 1995, National Academy of Sciences 2001).

Table 1. Continued.

Attribute	Extensive Traditional Farming Systems	Intensive Modern Farming Systems
Edge effects among habitats	Multiple and varied sorts of contacts among relatively different successional habitats. This may enhance interaction and exchange among local species assemblages (α-diversity), leading to increased landscape and regional diversity (γ-diversity).	Scarce and rather uniform edge contacts among relatively similar habitat types (low β-diversity) leading to low landscape or regional diversity (γ-diversity).
Rural/urban interface	Relatively stable over time; when they occur, land-use changes remain mostly rural (e.g. conversion of forests into pasture or agricultural fields; Ochoa-Gaona and González-Espinosa 2000).	Highly dynamic in space and time due to rapid land-use changes following escalating land price (Olson 1995, National Academy of Sciences 2001).

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Potential effects of transgenic maize on natural ecosystems

In this section we outline the main hypotheses to explain how transgenic maize may affect natural ecosystems. These effects depend on an interaction between transgenic maize and the natural ecosystem. This interaction could occur through the fluid boundaries between natural ecosystems and agro-ecosystems whereby organisms interact with (e.g. feeding, ovipositioning) and are exposed to transgenic maize directly, through movement of pollen or seeds into natural ecosystems resulting in exposure if an organism could be affected by pollen, seeds, gene flow, or volunteer plants.

We also review the current knowledge on the effects of transgenic crops on natural ecosystems with focus on Bt maize, due to available literature. Approaches to studying ecological effects have largely focused on quantifying lethal and sublethal effects of Bt toxin on insects. These studies have formed the basis for extrapolating what effects Bt crops may have on higher levels of ecologically relevant factors, such as populations, species, communities and ecosystems. To understand the net ecological effects of using Bt corn in Mexico, we discuss what comparisons to existing agricultural practices will produce the most informative assessments.

Hypotheses for transgenic crop effects on natural ecosystems with special emphasis on Bt maize

Previous reviews have outlined hypotheses for how transgenic crops may affect natural ecosystems (Wolfenbarger and Phifer 2000, Conner et al. 2003). We provide an overview of these and consider in detail what are most often referred to as non-target effects or unintended effects on organisms other than the pest(s) targeted. Assessment of non-target effects often focuses on highly charismatic species (i.e., butterflies) and agriculturally beneficial insects, such as predatory insects (green lacewing, ladybird beetles, *Orius* spp.). We focus on how effects from transgenic organisms, and Bt maize in particular, may have implications for biodiversity by discussing effects at progressively broader levels of ecological organization: individuals, populations, communities, and ecosystems. Two main categories of mechanisms for how effects on natural ecosystems may occur are (1) effects arising from a component of the transgenic organism (i.e., Bt toxin), and (2) effects correlated with the use of a transgenic organism (i.e., changes in farming practices associated with adoption of a transgenic crop).

At an individual level, impacts of significance could include lethal and sublethal effects (e.g., effects on development time, reproductive characteristics, morphological characteristics). In the case of Bt maize, mortality or sublethal effects would occur if Bt toxin is hazardous to the organism under study and if the organism contacts Bt toxin in the environment. Pathways for exposure would include direct contact (1) with Bt toxin in the transgenic plant, (2) with transgenic pollen containing Bt toxin, (2) with Bt toxin in soil (through plant decomposition or root exudates), and (4) indirect (or tri-trophic) contact through prey that have ingested and/or accumulated Bt toxin.

Impact on populations will depend on the consequence of effects on individuals and the variation of those effects. Sublethal effects on individuals may have impacts on the population growth rate, leading to small or inviable population sizes and to local extinction (Baveco and DeRoos

1996). Individual variability in sensitivity to Bt toxin could also produce population level effects if the loss of Bt-sensitive individuals from the population results in decreased genetic variation within populations or species. Loss of genetic variation increases population or species risk of extinction (Reed and Frankham 2003)

In turn, any impact on communities or ecosystems will depend on the role of an affected population or species in the ecosystem and whether that role is novel or redundant. The presence or absence of populations or species within a community or ecosystem may have significant impacts on biodiversity through ecological interactions and ecosystem dynamics (Whitham et al. 2003). For example, predator removal experiments demonstrate the concept of "keystone" predators whose presence or absence affects the diversity and abundance of other species (Navarrete and Menge 1996). Similarly, the removal or addition of a species or population may affect the function of an ecosystem, including nutrient dynamics and energy flow (Symstad et al. 2003). Lastly, if a susceptible species is rare or has small populations, any mortality or sublethal impacts on its populations may exacerbate an existing high risk of extinction.

Biodiversity and natural ecosystem effects that may arise from changes in agricultural practices associated with the adoption of transgenic crops

In the previous section, we emphasized hypotheses to explain potential impacts caused by the transgenic organism itself. However, given the past and predicted future negative impact of land use on biodiversity, equally important to consider are changes that may occur through the *use* of the transgenic organism. Ecosystems are not discrete units but rather represent interacting ecological entities and processes that vary in time and space. Natural ecosystems interact with agro-ecosystems at adjacent borders where community compositions change, or when soil or water runoff occurs due to site geography or soil properties. Therefore, any changes that the introduction of transgenic maize has on agricultural practices may have consequences (positive or negative) for natural ecosystems and the biodiversity associated with them.

For transgenic crops, these would include farming practices associated with their use. Adoption of transgenic organisms like Bt maize with insecticidal properties may alter practices associated with pest control, including type of pesticide, timing of application and number of applications. Any changes in pesticide use have the potential to alter impacts on non-target organisms and, in turn, alter impacts on populations, species, communities and ecosystems. For example, timing of herbicide application accounted for differences between fields planted with herbicide tolerant and conventional beets, spring oilseed rape, or maize in insect abundance and plant diversity in marginal habitats adjacent to fields (Brooks et al. 2003, Haughton et al. 2003, Heard et al. 2003). The direction of these patterns varied according to crop, with higher values associated with the herbicide tolerant corn due to the less efficient weed management that occurred within the fields, and lower values of insect abundance and plant diversity associated with better weed management within the fields of herbicide tolerant oilseed rape and beets. Field experiments have demonstrated that transgenic herbicide tolerant beets can be managed so that fields have higher biodiversity without compromising yield, although diversity measures were not conducted outside the fields so that the implications for adjacent habitats are not clear (Dewar et al. 2003).

In the United States, analyses indicate that adoption of Bt maize, targeting the European corn borer, has not resulted in changes in pesticide use, probably due to the fact that pesticides are

applied only in the event of a pest outbreak (Obrycki et al. 2001). In contrast, reports on Bt cotton indicate that insecticide use has decreased with its adoption (Ortman 2001, Pray et al. 2002). For cotton production overall in areas adopting Bt cotton, the number of insecticide applications and the use of insecticides that are most harmful to humans are declining (USEPA, 2001) and benefits on other vertebrates seem likely.

Adoption of transgenic herbicide tolerant soybeans in the U.S. is associated with increased conservation tillage practices because farmers can rely less on tillage as a tool for weed management. Tillage practices within a field affect surrounding natural ecosystems, and changes associated with adoption of transgenic crops could also produce ecological impacts by affecting contaminants transported by soil and water. Computer modeling indicated that the adoption of transgenic herbicide tolerant corn in the United States would reduce herbicide concentrations of atrazine and alachlor in vulnerable watersheds by replacing these with glyphosate and glufosinate (Wauchope et al. 2001).

Similarly, any habitat and landscape changes associated with adoption of transgenic crops also have the potential to affect individuals, populations and ecosystems. Changes in field sizes, in habitat fragmentation or in rates of habitat conversions from and to agriculture will alter habitat availability, an important determinant for impacts of agriculture on natural ecosystems. An evaluation of the possible effects of transgenic maize agriculture on ecosystem properties should consider not only "natural" systems (actually, they would be very rare in Mexico), as they may be connected by other systems affected to a variable extent by human activities. Effects of transgenic maize agriculture on local and regional biodiversity, on ecosystem resistance and resilience, and on the possible sustainability of the productive system are additional avenues of study to quantifying effects on biodiversity and ecosystems.

Current transgenic maize products and potential effects on Mexican biodiversity and natural ecosystems

In this section, we discuss in more detail how transgenic maize may impact natural ecosystems. We concentrate on synthesizing studies on Bt maize because the largest amount of research has been conducted with it. We begin the section by giving a brief overview of the product and properties of its insecticidal action.

Brief background on Bt toxin and its insecticidal activity

We synthesized the following from reviews on the diversity and mechanisms of action of toxins in *Bacillus thuringiensis* and also Bt crops from Schnepf et al. (1998) and deMaagd et al. (1999). *Bacillus thuringiensis* is a gram-positive, spore forming bacterium that occurs in many habitats and worldwide, including soil, insects and plant surfaces. During its growth cycle, *B. thuringiensis* produces one or more crystal proteins (Cry). These proteins result in insecticidal activity if a series of conditions occur: (1) dissolving of the protein in an alkaline environment within the insect midgut to release a protoxin; (2) truncation (trimming of the protoxins into an activated toxin; (3) binding of the active toxin to specific receptors on the epithial cell membranes of the midgut; and (4) formation of pores that ultimately kill the insect. *B. thuringiensis* strains and Cry genes exhibit a high degree of genetic diversity. Cry proteins are characterized according to their specificity to particular insect groups. In general, each Cry

protein has insecticidal activity for certain insect species among Orders such as Lepidoptera (butterflies and moth), Coleoptera (beetle), Diptera (flies), and Hymenoptera (bees and wasps). Other Bt strains have activity against mites, nematodes, flatworms and protozoa. Differences in active toxin and receptor interactions and in solubility in the insect midgut explain differences in the insecticidal properties among Cry proteins. *Bacillus thuringiensis* has been used as a biological pesticide since 1977 to control a variety of invertebrate pests for applications such as forestry, agriculture, and public health (e.g., mosquito and blackfly control). Transgenic Bt crops are engineered with genes that produce the activated toxin; therefore, the toxicity of an organism to Bt toxin from a transgenic crop may have different effects than those from microbial sprays.

Current transgenic maize products and their targets

Current transgenic maize products include insecticide-resistance to Lepidoptera and Coleoptera pests (both engineered with a Bt toxin gene) and herbicide tolerance to glyphosate (Round-up^{TM}). Given the specificity of Bt toxin to particular insect families and orders, insects within these orders would have the highest probability of exhibiting susceptibility to a transgenic maize event's particular Bt toxin(s). Lethal and sublethal effects to non-pest species in these insect orders could produce changes in biodiversity within these orders, depending on the susceptibility of other species within these orders to Bt toxin and their exposure to the toxin. Indirect effects on community and ecosystem diversity could occur if other more distantly related species or taxon groups were connected with these species through ecological relationships. For example, the abundance and diversity of the Lepidoptera could affect plant populations and species that depend upon butterflies and moths for pollination, or could affect populations and species of predators that prey upon butterflies and moths. Predatory species could be impacted in two ways by impacts on Lepidopteran species. Alterations in abundance or availability of prey could alter abundance or diversity of predators, or Bt toxin in prey species could affect individuals, populations and species of predators susceptible to Bt toxin.

Coleopterans are a diverse order with respect to numbers of species as well as ecological guilds represented. Because of this diversity, non-target effects on Coleoptera could have implications for nutrient cycling and decomposition, as well as plant pollination and abundance and diversity of prey and predator species depending upon them. If there were any exposure of Bt toxin to aquatic ecosystems near agricultural areas, aquatic species of Coleoptera could also be affected.

Thus far, we have emphasized the biological impacts that could arise if Bt toxin is a hazard to individuals and how these may, in turn, cause a cascade of effects at higher levels of ecological organization and relationship. The other important determinant in predicting the impact of Bt maize on natural ecosystems is its exposure to Bt toxin. For maize, Bt toxin may be present in its green tissue and its pollen and may also occur in the soil via transport from the roots and via plant decomposition. The adoption of Bt crops presents a series of pathways by which natural ecosystems could be exposed to Bt toxin in plant tissue, pollen or soil: (1) Bt maize plants, (2) hybrid plant between Bt maize and non-Bt maize, or (3) Bt maize to wild close relatives or their hybrids. Rates of pollen flow and gene flow will influence the extent of area exposed to Bt toxin. Given the validity of species-area curves, when pollen flow rates and/or gene flow rates are high, the number of species exposed will increase. Similarly, field dimensions and area of Bt crops will affect the extent to which natural ecosystems interact with Bt maize and the amount of available pollen or seeds for transport into natural ecosystems.

Overlap of natural ecosystems with wild relatives of maize and maize production

The geographical distribution and range of habitats occupied by maize and its close relatives is not yet settled. New discoveries are to be expected from further botanical exploration in little known regions of Mexico and Central America (Doebley 1990). According to Doebley (2003) there are four recognized species of *Zea* (*Z. diploperennis*, *Z. luxurians* , *Z. mays*, and *Z. perennis*), and four subspecies in *Zea mays* (*Z. m. huehuetenangensis*, *Z. m. mays*, *Z. m. mexicana*, and *Z. m. parviglumis*). Recently, Iltis and Benz (2000) have treated populations of *Z. luxurians* from Nicaragua as a separate species, *Zea nicaraguensis*.

Zea mays ssp. *parviglumis*, a wild annual teosinte (Balsas teosinte), occurs in southern Nayarit, Jalisco, Colima, Michoacán, the State of Mexico, Morelos, Guerrero, and southern Oaxaca; Doebley 1990, Sánchez-González et al. 1998). It mostly grows as part of native vegetation at lower elevations (400-1,800 m) in warm humid and sub-humid climates; rarely hybridizes with maize as it grows away from corn fields in prairies, rocky cliffs, and roadsides (Wilkes 1977, Doebley 1990). Other annual teosinte, *Z. mays* ssp. *mexicana*, grows as a weed in many maize fields in temperate humid and sub-humid climates of central and northern Mexico (1,700-2,600 m), and frequently hybridizes with maize. This subspecies distributes from the Chihuahua-Sonora border (Papigochic) and the Nabogame Valley of Chihuahua (1,850 m) southwards along the Sierra Madre Occidental (Durango) to the plains and valleys of the central highlands of Mexico (northern Michoacán, Guanajuato, eastern Jalisco, the State of Mexico and Distrito Federal, at 1,800-2,600 m elevation; Sánchez González et al. 1998). A more distantly related annual teosinte (*Z. mays* ssp. *huehuetenangensis*) occurs in the higher basin of the Grijalva River in W Guatemala (900-1,650 m), very close to the Mexican border (Frontera Comalapa and Chicomuselo, in Chiapas).

Other forms of teosinte that may hybridize with maize (Doebley 1990), but have been found not to be involved in its origin (Matsuoka et al. 2002) are: *Zea diploperennis* (only known from the Sierra de Manantlán, Jalisco, at 1,400-2,400 m elevation), *Z. perennis*, (only known from the N slopes of Volcán de Colima, Jalisco, at 1,500-2,000 m), *Z. luxurians*, from SE Guatemala and Honduras, from sea level up to 1,100 m (in Mexico, it has been collected only once in southern Oaxaca in 1845). *Zea nicaraguensis* is considered the most primitive of the annual teosintes and is native to lowland habitats near the Gulf of Fonseca in Nicaragua (Iltis and Benz 2000). The basal or most primitive forms of maize are identified in materials from the highlands (above 1,800 m), overlapping with subsp. *parviglumis* between the states of Jalisco and Oaxaca. The oldest archaeological record of maize (about 6,250 years ago) is known from the Guilá Naquitz cave in the central highlands of Oaxaca (1,920 m elevation; Piperno and Flannery 2001).

Conceding that further botanical explorations are still badly needed, the available recent literature on taxonomy and phytogeography of *Zea*, and on the evolution and domestication of maize based on molecular genetics, cytogenetics, and archaeological evidence, provide a basis for some outlooks. Maize, wild teosintes, and species in close genera (e.g., *Tripsacum* and other native taxa in the tribe Tripsacinae; Davidse and Pohl 1994, Kellogg 1998) broadly overlap in their ecological and geographical distributions. This sympatry over an enormous extent of land and diversity of habitats, and the lack of barriers to hybridization among maize and teosintes, provide ample opportunities for gene flow between them although not always in both directions and among all taxa (Doebley 1990). Potential ecosystem level effects from transgenic maize

could occur wherever introgression may produce novel genotypes that may interact with other elements of the biota (e.g. microorganisms, weeds and insects) which may afterwards behave as invasive organisms in unexpected ways (Wolfenbarger and Phifer 2000, Conner et al. 2003). (Likelihood of this is topic considered by authors of Chapter 3.)

Comparison of transgenic maize with existing maize production in Mexico

The net impact of transgenic maize compared to existing agricultural practices on natural ecosystems will depend on whether maize fields are a source of biodiversity, or a factor maintaining ecosystem dynamics for interacting natural ecosystems, and on the relative consequences of changing existing maize farming practices and values on natural ecosystems surrounding fields (Table 1). None of these hypotheses is specific to Mexico, but rather these have been generated before and after the commercialization of Bt maize events in the United States and Canada. However, as many authors have stressed in the past and continue to stress, the impacts of transgenic maize, or any transgenic organisms, must be evaluated on a case-by-case basis. In part this is due to differences among the transgenic organism, but equally important are the differences in the environments receiving the introduction of a transgenic organism. In the next section we review existing literature on impacts of Bt maize on natural ecosystems.

Data on biological effects of Bt maize

Four delivery routes for Bt toxin mimic what may occur for organisms living in natural ecosystems: (1) ingestion of green tissue, (2) ingestion of Bt maize pollen, (3) ingestion of prey items containing Bt toxin, and (4) contact with or ingestion of soil containing Bt toxin. The majority of the available studies have been designed to measure impacts on individuals; however, some measures of population-level effects have been conducted.

Ingestion of tissue

The effects of Bt maize tissue on individual herbivores are variable among species. When fed maize leaves with and without Bt toxin (Cry1Ab), no effect on survival, development time or mass was detected in individuals of an aphid species (*Rhopalosiphum padi*) and those of a species of spider mite (*Tetranychus urticae*) (Dutton et al. 2002). However, for a moth species (*Spodoptera littoralis*), significant differences occurred (Dutton et al. 2003). Fewer individuals that fed on Bt plants survived, and they had longer development times. Quantification of the average amount of Bt toxin in each herbivore indicated the highest levels in the spider mite (2.5 mcg/g) and followed by a lower level in the moth (0.72 mcg/g). Tiny amounts were detected in the aphids (0.02 mcg/g); such levels in the aphid are not surprising because they feed on phloem and because this tissue does not contain Bt toxin (Raps et al. 2001).

In addition to ingestion of green tissue, other organisms contact Bt maize and its toxin through decomposition processes. Over a 20-day period the decomposer *Porcellio scaber* (a woodlouse) fed significantly less on transgenic Bt corn than non-Bt corn although considerable variation among the two transgenic and six non-transgenic corn varieties occurred. Bt toxin (Cry1Ab) was detected in the body and feces of *P. scaber* (Wandeler et al. 2002). In a 200-day study, no lethal effects were detected in adult or immature earthworms (*Lumbricus terrestris*), and there were no

significant differences in weight between individuals fed Bt corn litter (Bt11) and non-Bt corn litter (Zwahlen et al. 2003).

Ingestion of Bt corn pollen

Monarch butterflies (*Danaus plexippus*) are the best-studied example of how Bt corn may affect a non-target organism under field and laboratory conditions. Studies were conducted with three Bt corn events (Bt11, Mon 810 and event 176 [no longer commercially available]) that differ in the amount of toxin present in their tissues. In laboratory and field studies with low doses of Bt corn pollen, survival of monarch larvae on milkweed (*Aesclepias syriaca*) or black swallowtails (*Papilio polyxenes*) on wild parsnip (*Pastinaca sativa*) was not affected by the presence Bt corn pollen from Bt11 or Mon 810 (Hellmich et al. 2001, Stanley-Horn et al. 2001, Wraight et al. 2000). At higher Bt corn pollen doses, the species-specific effects may vary. With Bt corn pollen from event 176 on larval host plants, monarch larvae had higher mortality than when fed leaves with non-Bt corn pollen (Losey et al. 1999), a result confirmed in corn fields (Zangerl et al. 2001). Sublethal effects of Bt corn pollen from event 176 were observed on Monarch larvae that fed on host plant leaves with low pollen densities (5-10 grains cm^{-2}) (Stanley-Horn et al. 2001). Mortality of swallowtail larvae due to Bt toxin occurred only at high doses, such as those occurring with event 176 within corn fields and at their boundary (Zangerl et al. 2001). In contrast, no effects of Bt11 or event 176 on larval mortality were reported in studies on the milkweed tiger moth (*Euchatias egle*) (Jesse and Obrycki 2002). In the case of event 176, the higher mortality effects were related to the higher toxin production. Event 176 is no longer available commercially, but the research illustrates a broader point than the conclusion that Event 176 will cause harm. Namely, transformation events of the same gene may vary in their ecological impacts due to species sensitivity as well as opportunity for exposure. For example, in the northern U.S. range of monarchs (Minnesota, Wisconsin) significant overlaps occur between larval development and pollen shed; whereas less overlaps occur in the southern part of the range (Oberhauser et al. 2001). For species with more restricted distribution than the monarch, results from risk assessment may produce different conclusions given the greater opportunity for exposure.

Contact with or ingestion of soil containing Bt toxin

Roots of Bt corn exude Bt toxin (Saxena and Stotzky 2000), creating an additional pathway by which soil organisms may be exposed. Experiments report varying amounts of time for how long Bt toxin remains in the soil, due to differences in soil composition used and conditions under which the decay is studied (Stotzky 2001; Zwahlen et al. 2003a). No effects on earthworms, nematodes, protozoa, bacteria or fungi in soil were detected using total abundance measures (Saxena and Stotzky 2001). Similarly, Zwahlen et al. (2003b) reported no effects on mortality of earthworms after 200 days exposure to soil containing Bt corn, but also stressed the importance of quantifying sublethal effects that may occur due to longer or earlier exposure to Bt toxin.

Ingestion of prey containing Bt toxin: Prey-mediated effects

In experiments with small plots (0.405 ha), no significant differences in predation or parasitism rates of European corn borer eggs were observed between Bt corn and conventional corn (Orr and Landis 1997), indicating no observable effects on insect predators and parasites with a small-

scale release. Experiments comparing the effects of prey fed Bt corn silk or non-Bt corn silk on the predator *Orius insidiosus* detected no effects on development time, body weight, body length or mortality (Al-Deeb et al. 2001). Similarly, no effects on mortality or development time in *O. majusculus* were detected when prey fed on Bt and non-Bt corn plants (Zwahlen et al. 2000)

Hilbeck et al. (1998, 1999) reported higher mortality of the agriculturally beneficial green lacewing (*Chrysoperla carnea*) used in treatments with prey fed Bt toxin or Bt corn compared with respective control treatments. Dutton et al. (2002) corroborated these results and reported similar differences in mortality using the same prey species, *Spodoptera littoralis*. Romeis et al. (2004) conducted experiments using a bioassay that allowed them to test the direct effects of Bt toxin on green lacewing larvae, and their results indicated that negative effects on mortality were mediated by prey-quality and not by direct effects of Bt toxin. Two other prey species tested by Dutton et al. (2002), included the aphid (*R. padi*) and the spider mite (*T. urticae*), did not produce effects on mortality in *C. carnea*. Quantification of the amount of Bt toxin in the treatments and controls confirmed that aphids do not ingest Bt toxin when feeding upon phloem. However, high levels of Bt toxin were present in *T. urticae*. Further experiments indicated that *C. carnea* may avoid *S. littoralis* that have fed on Bt corn but do not discriminate between *R. padi* that have fed on Bt or non-Bt corn (Meier and Hilbeck 2001). *C. carnea* also choose *R. padi* as prey over *S. littoralis*, suggesting that prey availability as well as preferences will affect any impacts (Meier and Hilbeck 2001). These results highlight that tri-trophic or prey-mediated effects will vary according to prey species, predator species, and an interaction between the two.

Bt corn engineered to target the Coleopteran corn rootworm pests, *Diabrotica* spp., was commercialized in 2003 the United States. These events are engineered with a different type of Cry protein (Cry3Bb) from the Bt corn targeting lepidopterans. Fewer non-target studies exist in the literature, but two published reports indicate no adverse effects. For example, no prey-mediated effects on *Coleomegilla maculata*, a predatory beetle, were observed when comparing survival, larval development, and reproductive capacity of individuals among diets treatments containing or lacking Bt corn pollen (Duan et al. 2002, Lundgren and Wiedenmann 2002). Like the case with Lepidoptera, the diversity and species of Coleoptera exposed in Mexico will differ. With few related species studied, results from North America should be viewed as having limited applicability to Mexican ecosystems.

Studies with population level endpoints

Comparing insect abundance between Bt corn and non-Bt cornfields has detected no consistent differences. In a study in Ohio a single non-Bt cornfield had a significantly higher number of a species of rove beetles, whereas a Bt field had significantly more *Orius* spp. The study concluded that few negative effects on a group of 15 non-target arthropods could be directly associated with transgenic corn, although other relevant environmental or ecological factors were not incorporated into the analyses (Jasinski et al. 2003). Plot-level studies detected no significant differences in abundance of green lacewings, although the authors point out the need for studies on larger fields because of high between-year variability and small plot sizes (Pilcher et al. 1997). In comparisons of Bt- and non-transgenic sweet corn, there were significantly higher numbers of *Coleomegilla maculata* larvae in non-Bt treatments in open and caged plots and higher adult densities in non-Bt corn caged plots (Wold et al. 2001). No additional differences in species diversity of beneficial insects were detected although, once again, designs and sample

sizes with higher statistical power to detect relatively subtle effects were recommended in the conclusions of the study.

Ecological impacts of changes in farming practices: Farm Scale Evaluations as an example

Mexico is one of the countries where more land is used to provide maize as a staple food for a population of ca. 100 million people (OECD 2003). More than 75% of maize in Mexico is produced by small peasants that use rather traditional farming systems (Turrent-Fernández et al. in OECD 2003). Maize is typically sown with a scattered spatial arrangement across the corn field that provides opportunities for establishing other associated crops as beans and squash. Furthermore, this relatively open spatial arrangement of the crop plants allows for the persistence of populations of a large number of weed species in cornfields. Avila Diaz (1992) found that species richness of this plant guild in Mexican in a typical agricultural field in the Tarascan Plateau of Michoacán is generally high (26-94 species) but variable. Villegas (1970) reported that 232 weed species were present in the agroecosystems of the southern region of the Basin of Mexico. Espinosa-García and Sarukhán (1997) include 159 species and intraspecific taxa in their weed catalog of the Basin of Mexico. In the central highlands of Michoacán (1,900-2,000 m), Avila Díaz (1992) reports on 78 species belonging to 67 genera and 28 botanical families in inventories from 10 rainfed maize fields. Fuentes-Delgado et al. (1985) found the same total number of 37 weed species in 19 botanical families over two yearly cycles with maize and bean at Chapingo, México. Vieyra-Odilón and Vibrans (2001) found 74 weed species in maize fields in the Valley of Toluca at 2,540 m elevation.

It has long been recognized in traditional rural areas of Mexico that this group of species are not "weeds" in the strict and rather narrow meaning this term receives in North America and Europe, where usually only the detrimental effects on main crop plants are used to define and segregate them. In Mexican traditional agricultural regions, even if yield reductions of the main crops occur (see Vieyra-Odilón and Vibrans 2001), their possible detrimental effects on crop yields can usually be compensated by cash income after being sold in near markets, because of diet enrichment through direct consumption by the farmer´s family, or because of other household uses (Nations and Nigh 1980, Azurdia 1981, Hernández Xolocotzi 1985a, Bye 1981, 1993, Vieyra-Odilón and Vibrans 2001). Therefore, the relationships of Mexican poor peasants with their "weeds" may be quite complex, and they represent a rich genetic resource on which selection towards domestication may take place. For example, it has been documented that some "agrestic" (*arvenses*) species in the Solanaceae are still selected by Tlaxcalan farmers for their edible fruits (Williams and Hernández Xolocotzi 1996). Notwithstanding this high biological and cultural richness associated to spontaneous plant species in traditional agricultural systems of Mexico, the ecology of weeds has been little studied. Most detailed studies refer to ethnobotanical issues of a few groups of species (e.g., Bye 1981, 1993, Mapes et al. 1997, Vieyra-Odilón and Vibrans 2001). It still remains to be studied in Mexican agroecosystems the structure, changing composition, stability and resilience of this highly valued community of wild plants, as well as their cascading effects, in farming systems that vary in their number and degree of technological innovations — including the possible adoption of transgenic crops.

Conceding that an adequate baseline for comparison with Mexican agroecosystems is not available, we use an example from studies on herbicide tolerant crops to discuss the importance of considering changes in farming practices. Effects of using herbicide-tolerant crops were

examined experimentally in the Farm Scale Evaluations Project (FSEs) conducted in the United Kingdom (Freckleton et al. 2003). These studies represent the best example to date of the ecological consequences of changes in farming practices associated with adoption of transgenic crops. Researchers reported significant changes in abundances and diversity of invertebrates associated with the management of genetically engineered herbicide-tolerant beets, oilseed rape, and corn, both within cropland and in habitats adjacent to fields (Brooks et al. 2003, Haughton et al. 2003, Roy et al. 2003). These studies underscore the value of a case-by-case approach because the direction and magnitude of effects on invertebrates varied among the crops studied. For example, most decreases in invertebrate taxa were associated with genetically engineered herbicide-tolerant beet and oilseed rape, and most increases were associated with genetically engineered herbicide-tolerant corn. Changes in invertebrate abundance were associated with more effective weed control in fields planted with genetically engineered, herbicide tolerant beets and oilseed rape (Heard et al. 2003). In particular, timing of herbicide spraying affected weed control and therefore the associated invertebrate communities. Invertebrate detritivores increased in fields of all three genetically engineered herbicide-tolerant crops, which was attributed to greater biomass of dead weeds in these fields (Hawes et al. 2003). The design of the Farm Scale Evaluations most likely underestimated ecological effects because cumulative effects were not included and because a split-plot design could reduce the possibility of detecting scale effects. These results highlighted earlier discussions about the impacts of "clean" agricultural fields and field margins on habitat that supports other organisms, including birds (Watkinson et al. 2000).

Summary of known impacts and unknown impacts

For a few species (e.g., monarch butterflies, green lacewings, *Orius spp*) we have accumulated information to quantify the hazard to individuals of ingesting Bt toxin from Bt corn directly or via prey, and to quantify the exposure for specific Bt corn events. Collectively, these studies have largely reported no differences between Bt corn and non-Bt corn. Population-level measures have detected no consistent differences between Bt cornfields and non-Bt cornfields; in the case of transgenic sweet corn, a product that is associated with large decreases in insecticide use, significant differences in insect numbers were observed in non-Bt cornfields. Any strong conclusions from the population-level data we currently have would underestimate the potential for population level effects given the magnitude of effect detectable, the number of species tested and the short time frame of the existing studies. Two notable exceptions to the lack of differences exist. First, Bt toxin from Bt corn event 176 (no longer commercially available) decreased survival of two butterfly species (monarchs and black swallowtails). Second, results from experiments with green lacewings varied according to prey species used. Collectively, these experiments have demonstrated the challenge of testing whether and how transgenic corn affects individuals. They also highlight that habitat-specific factors, such as prey availability, will influence whether or not effects will occur. Furthermore, ecological consequences may vary according to cultivars and transformation events as well as the species-specific variability in the effects of a toxin.

The data applied to assessing Bt corn in the United States is not necessarily transferable to natural ecosystems outside the United States or sufficient to predict consequences on biodiversity and ecosystems function. Ecological processes may remain similar, but the players, their roles, and the scenarios (agricultural landscapes) may change dramatically. The available research on

Bt corn represents the largest body of work on the potential for ecological impacts of transgenic organisms; however, we have little information to use to predict effects on ecosystems compared with the biodiversity in Mexico that will affect ecosystem functioning. From the available data, we have studies that have supported an in-depth risk assessment of the effects of Bt corn on monarchs (Sears et al. 2001). No adverse effects on monarch populations are predicted given the level of exposure to Bt pollen from two transgenic events (Bt 11 and Mon810). We have information on the effects in the field for few other species: three native butterfly species; a small number of agriculturally-beneficial organisms (green lacewings, ladybird beetle, minute pirate bugs), and on prey species of insect predators (aphids). In terms of ecological function, we have information on a few representative, often non-native species of pollinators, predators, decomposers and herbivores. We lack information testing whether any changes in biodiversity of particular taxa, such as butterflies and moths, have occurred as a result of introducing Bt corn in the United States. Studies on these higher order relationships may be most informative for assessing effects in other ecosystems. In the United States, 132 plant species in 33 families occur in maize agroecosystems, and 229 lepidopteran species in 21 families feed on these plants (Losey et al. 2003). Letourneau et al. (2002) compiled a list of 376 lepidopteran species that feed on *Zea mays*; published toxicity studies on susceptibility to Bt endotoxin were available for 11 of these. Given the known species to species variation in susceptibility even within target orders, predictions on the consequences on lepidopteran diversity remain tenuous. As herbivores and pollinators, any positive or negative effects on lepidopterans would be expected to have subsequent effects on other organisms and processes with the ecosystem, given the varied ecological roles that lepidopterans play (reviewed in Letourneau et al. 2002).

The expertise with which to assess impacts of changes in biodiversity on natural ecosystems is under development, and predictions of how transgenic maize will affect natural ecosystems in Mexico will be accompanied with uncertainty. However, that uncertainty can be reduced by considering and incorporating the ecological context in which transgenic maize will be introduced. Choice of informative species, choice of realistic or field-based test environments, and choice of experimental designs with the power to detect smaller differences can reduce some of the uncertainty associated with an assessment. Predicting the relative impacts will require a regional approach given the diversity of agricultural practices in Mexico and the regional variation in species composition and abundance. For existing farming practices, factors that will be important to consider include: how adoption of transgenic maize may affect chemical inputs, such as insecticides; what net consequences these insecticides have on natural ecosystems (as opposed to the in-field effects); and how will the adoption of transgenic crops affect conversion of natural ecosystems to farmlands.

In summary, in response to the question, "Will the introduction of transgenic maize have a positive or negative effect on natural ecosystems in Mexico?" we answer the following. "Existing data on transgenic maize will not address this question sufficiently. We have strong concerns that there is limited applicability of data collected thus far to address the impacts of transgenic maize on biodiversity and natural ecosystems in Mexico. The ecological diversity of agroecosystems where maize is cultivated in Mexico is substantially higher than in other countries that have adopted transgenic maize, and the consequences of any changes in the biodiversity are not yet predictable from studies of ecosystem function and biodiversity loss."

Areas in which there are unresolved and/or controversial issues

Unresolved issues

We join a growing number of researchers who have highlighted the lack of adequate data with which to assess the relative impact of transgenic crops on natural ecosystems and, perhaps, even broader the lack of an approach with which to assess effects on biodiversity and natural ecosystems (*sensu* Groot and Dicke 2002, Losey et al. 2003, Letourneau et al. 2002). The effects of transgenic maize in Mexico will depend on the toxicity of any pollen to non-target organisms, the movement of transgenic pollen to natural ecosystems, transgene flow to natural ecosystems, what ecological impacts the use of the transgenic maize has on natural ecosystems and landscapes, and the ecological role of the non-target organism in the ecosystem. Certainly, the key question is the relative impact of transgenic maize on natural ecosystems when compared to existing or alternative agricultural practices.

Scientific Controversies

Recent reviews of studies on the ecosystems consequences of biodiversity loss stress the challenges associated with predicting these relationships for a given ecosystem. At least four major questions in this area remain unresolved:

1. What species and how many are the best indicators of ecosystem processes and function?

2. What ecological interactions best represent ecosystem dynamics necessary for function?

3. What ecological impacts will changes in biodiversity have on ecosystems?

4. What amount of biodiversity is necessary for ecosystem stability (i.e., how much redundancy or resiliency does an ecosystem have)?

Uncertainty remains over what magnitude (50%, 25% 10%, 5%) of change between experimental and control individuals in turn leads to effects on populations, species, or higher order interactions. Experimental designs of small-scale, pre-commercial field experiments are not sufficiently sensitive enough to detect small or moderate effects on non-target individuals from a transgenic organism (Marvier 2003). Small-scale studies will readily detect order-of-magnitude differences in an ecological effect, but less dramatic effects will be difficult to document due to variability among replicates (Andow 2003). Much more powerful designs are needed for detecting small but cumulative effects that would influence ecosystem composition and functioning.

Unresolved or undefined policy issues

Value is placed on ecosystems because some members of society place economic or intrinsic value on their existence. The value placed on ecosystems is likely to vary among stakeholder groups and can be a significant unknown in evaluating the value of ecosystems. Further, the value placed on specific ecosystems services will also vary among groups. The two extremes of attributes associated with traditional farming systems and intensive modern farming practices Table 1) represent examples of what respective farmers that might adopt transgenic maize would value from natural ecosystems — if only as a starting point for analysis. In the United States, testing required prior to commercialization emphasize the value placed on pollination services,

pest species population control, and soil quality. The controversy over whether Bt maize would harm monarch butterflies also highlighted the value placed on "charismatic" species. How to assign these values, which components of ecosystems these values would focus research on, and the opinions of which stakeholder groups to consider, combine to make this a significant, unresolved policy issue for evaluating transgenic crops.

Easier to define are the goods and services associated with ecosystems. These include tangible goods provided by ecosystems such as food and fiber, and services such as air and water purification. In the context of the neotropics, some ecosystems are also repositories of valued genes associated with the progenitors of valuable crop varieties, such as landraces of maize. The value of ecosystems to existing agricultural practices extends to the provision of pollinators for some crops, and the role of native ecosystems in water and nutrient cycles. These values are typically degraded by human activities such as agriculture. In order to understand impacts of transgenic maize in Mexico, multiple reference points for comparison may be necessary, due to regional variation in agricultural practices as well as the cultural values associated with particular agricultural practices.

How to incorporate uncertainty into analysis of risks and benefits is a difficulty inherent in risk assessment although not a specific outcome of addressing transgenic crop varieties. Stakeholders will most likely vary in what value should be placed on uncertain outcomes; incorporating these is an important component of the decision-making process. A solution to this dilemma does not present itself, but the issue is being considered in conjunction with a variety of issues including transgenic crops, invasive species, and global climate change.

Priority topics where more research is needed to improve our understanding of the issues being addressed.

The link between biodiversity and ecosystem functioning

Biological diversity is known to be important in the structure and function of ecosystems (Kinzig et al. 2001). However, the exact role diversity plays in ecosystems is hotly debated (Loreau et al. 2001, 2002). Much of the current debate revolves around experiments on highly simplified ecosystems. A major challenge for science is to develop experimental designs that are capable of detecting differences in ecosystem function in more complicated systems. This is a critical stage to apply this information to areas like Mexico where the level of biological diversity is substantially higher than that considered in most mesocosm experiments or in-field experiments conducted in temperate latitudes.

Identifying species within ecosystems in Mexico that should be the focus for predicting ecosystem effects

A prioritization of species at highest risk and of highest value will facilitate studies assessing ecological effects. Careful thought and consideration must be given to what organisms are used and how many species are used to quantify ecological impacts. Relying solely on existing studies conducted in the U.S. and Europe would underestimate impacts on natural ecosystems due to large scientific and ecological uncertainty associated with determining whether these existing studies are representative of Mexican ecosystems.

Promoting a regional approach to defining and assessing the ecological impacts of introducing transgenic maize

Mexico contains a rich local and regional level of cultural and biological diversity, both of which will contribute to natural ecosystems and agroecosystems. Given the diversity also of local landraces of maize, a regional (and possibly even smaller scale) approach will be need to effectively address whether negative impacts of introducing transgenic maize will occur. Comparisons to existing agricultural practices for maize production will produce information for a science-based approach to evaluating impacts of transgenic maize on natural ecosystems.

Presenting and discussing uncertainty of data used in assessments for predicting effects on biodiversity and natural ecosystems

For experiments used in assessing ecological impacts of transgenic organism on natural ecosystems, researchers should calculate the magnitude of difference that could be detected and discuss its ecological significance for natural ecosystems. Such an approach will highlight areas where uncertainty in an outcome may be high or low, allowing this factor to be available for a decision-making process.

Conclusions

In summary, we underscore the need for ecologically-relevant experiments designed for assessing the effects of transgenic maize in Mexican ecosystems. Concomitantly, we highlight the uncertainty associated with applying the limited number of existing studies conducted in the U.S. and Europe to Mexico, a country noted for its high level of cultural, biological, and ecosystem diversity. In order to strengthen the process of assessing the effects of transgenic maize, experiments focused on biologically- and culturally-valued species in Mexico that may be affected by the introduction of GM maize will produce the most useful information. An understanding of how these species contribute to and affect ecosystem function will provide necessary information with which to predict effects on biodiversity. Lastly, given that the effects of transgenic maize could vary according to the local and regional variation in biodiversity and agricultural practices, a science-based approach to predicting ecosystem and biodiversity effects assessments will require a scale reflecting regional variation in biodiversity and ecosystems.

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